


Bone density, bone geometry and bone development in young men

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The importance of pubertal timing and fracture history

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UNIVERSITY OF GOTHENBURG

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Cover illustration: Image of the right hip assessed by DXA (Hologic Discovery W)

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ABSTRACT

Background and objective: Peak bone mass, the maximal bone mass attained in young adulthood, is an important factor of the lifetime risk of developing osteoporosis. The aim of this thesis was to study the development of bone mineral density (BMD) and bone geometry around the time of peak bone mass in men, and also to investigate the association between pubertal timing, fracture history, bone turnover markers and BMD and bone geometry in young men.

Methods: The studies included in the thesis were performed within the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study, a well-characterized population-based cohort including 1068 men between 18-20 years of age at baseline. At baseline and follow-up five years later, measurements of bone density, bone mass and bone geometry were assessed with dual energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT). Blood samples were drawn to measure bone turnover markers. A self-administered questionnaire was used to collect information about physical activity, nutritional intake, smoking and previous fracture. Reported fractures were verified in X-ray registers.

Results: Previous fracture was associated with lower BMD at age 19, and especially with reduced trabecular volumetric BMD (vBMD) of the radius. Between 19 and 24 years of age, lumbar spine areal BMD (aBMD) increased while femoral neck aBMD decreased. Radius aBMD increased, due to increased cortical thickness and continuing mineralization. Men with late puberty had larger gains in aBMD, vBMD, and bone size, reflecting a catch up in bone acquisition in young adulthood in men with late puberty. A high level of osteocalcin (a bone turnover marker) was associated with larger gains in aBMD, vBMD, and bone size between 19 and 24 years.

Conclusion: In young adult men between 19 and 24 years, aBMD of the lumbar spine and the radius continued to increase, while aBMD of the femoral neck already started to decrease. Late puberty and high level of osteocalcin were associated with greater increases in aBMD, vBMD and bone size during this period. A previous fracture was a risk factor for low BMD in young men.

Keywords: peak bone mass, bone mineral density, bone development, fracture, young adulthood, men

SAMMANFATTNING PÅ SVENSKA

Bakgrund: Den maximala benmassan (peak bone mass) som uppnås i ung vuxen ålder, är en viktig faktor för risken att senare i livet drabbas av benskörhet (osteoporos) och därmed ökad risk för benbrott (frakturer). En hög maximal benmassa minskar risken för benskörhet.

Frågeställning: I den här avhandlingen studerades utvecklingen av benmassa, bentäthet och benstorlek hos unga män vid tiden för maximal benmassa. Vi undersökte om denna utveckling skilde sig åt beroende på tidpunkt för pubertet och beroende på nivåer av benomsättningsmarkörer som kan mätas i blodprov. Vi studerade också sambandet mellan fraktur under uppväxten och bentäthet och benstorlek i ung vuxen ålder.

Metod: Delstudierna som ingår i avhandlingen utfördes inom en välkarakteriserad populationsbaserad kohort bestående av 1068 unga män som vid studiens start var mellan 18 och 20 år gamla (the Gothenburg Obesity and Osteoporosis Determinants (GOOD) Study). Vid studiens början och vid uppföljningen fem år senare utfördes mätningar av bentäthet, benmassa och benstorlek med hjälp av dubbelfotonröntgenabsorptiometri (DXA) och perifer kvantitativ datortomografi (pQCT). Blodprov för mätning av benomsättningsmarkörer togs. Information om fysisk aktivitet, näringsintag, rökning och fraktur förekomst samlades in med hjälp av frågeformulär. Förekomst av frakturer verifierades genom sökning i röntgenarkiv.

Resultat: Mellan 19 och 24 års ålder minskade bentätheten i höften medan den ökade i ländryggen och radius (underarmen). De män som kommit i puberteten sent hade lägre bentäthet vid 19 års ålder, men ökade mer i bentäthet mellan 19 och 24 års ålder. Vid 24 års ålder hade män med sen pubertet kvarstående lägre bentäthet i radius, men inte i höft eller ländrygg. Hög nivå av benomsättningsmarkören osteocalcin vid 19 års ålder var kopplad till större ökning i bentäthet och benmassa mellan 19 och 24 års ålder. De män som haft en fraktur under uppväxten hade vid 19 års ålder lägre bentäthet.

Slutsatser: I ung vuxen ålder (19-24 år) sågs hos män en fortsatt ökning av bentäthet i ländrygg och radius, medan bentätheten i höften började sjunka. Sen pubertet och hög nivå av osteocalcin var kopplat till större ökning av bentäthet och benstorlek under den här perioden. Att ha haft en fraktur under uppväxten var en riskfaktor för låg bentäthet hos unga män.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. Darelid A*, Ohlsson C*, Rudäng R, Kindblom JM, Mellström D, Lorentzon M. Trabecular volumetric bone mineral density is associated with previous fracture during childhood and adolescence in males: the GOOD study. *The Journal of Bone and Mineral Research*, March 2010; 25(3):537-544.
- II. Ohlsson C*, Darelid A*, Nilsson M, Melin J, Mellström D, Lorentzon M. Cortical consolidation due to increased mineralization and endosteal contraction in young adult men: a five year longitudinal study. *The Journal of Clinical Endocrinology and Metabolism*, July 2011; 96(7):2262-2269.
- III. Darelid A, Ohlsson C, Nilsson M, Kindblom JM, Mellström D, Lorentzon M. Catch up in bone acquisition in young adult men with late normal puberty. *The Journal of Bone and Mineral Research*, October 2012; 27(10):2198-2207.
- IV. Darelid A, Nilsson M, Kindblom JM, Mellström D, Ohlsson C, Lorentzon M. Bone turnover markers predict bone mass development in young adult men: a five year longitudinal study. *The Journal of Clinical Endocrinology and Metabolism*, January 2015; epub before print.

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ABBREVIATIONS

aBMD	Areal bone mineral density
BMC	Bone mineral content
BMD	Bone mineral density
BTM	Bone turnover marker
CSA	Cross sectional area
DXA	Dual energy X-ray absorptiometry
EC	Endosteal circumference
GOOD	Gothenburg Osteoporosis and Obesity Determinants
HR-pQCT	High resolution pQCT
NTX	N-terminal telopeptide of type 1 collagen
OC	Osteocalcin
PBM	Peak bone mass
PC	Periosteal circumference
PHV	Peak height velocity
pQCT	Peripheral quantitative computed tomography
RCT	Randomised controlled trial
SD	Standard deviation
μ SV	Microsievert
vBMD	Volumetric bone mineral density
WHO	World health organization

DEFINITIONS IN SHORT

Bone turnover markers	Substances that reflect bone formation or bone resorption and can be measured in samples of blood or urine
Osteoporosis	A disease characterized by low bone density and microarchitectural deterioration of bone tissue, which leads to bone fragility and increased risk of fracture
Peak bone mass	The maximal attained bone mass in life
Peak height velocity	The most rapid longitudinal growth

1 INTRODUCTION

1.1 The skeleton and its functions

The human skeleton consists of over 200 bones, arranged in a sophisticated manner in order to protect vital organs and to allow movement of the body. The axial skeleton (skull, vertebrae, rib cage) forms a protective shell around the brain, spinal cord and inner organs, while the appendicular skeleton (the upper and lower limbs) is crucial for motion. Bones serve as attachment sites for muscles and ligaments, thereby enabling the body to move. The skeleton is also a reservoir for minerals such as calcium and phosphate, harbors hematopoiesis, and is an endocrine organ producing substances such as osteocalcin, which influence both bone cells and other cells.

1.2 Bone structure

The skeleton is comprised of long (tubular) bones, such as humerus, radius, femur and tibia, and flat bones, such as the skull, sternum, scapula and ileum (1). Bones have a dense outer shell, called cortex, which is composed of compact bone called cortical bone. Cortical bone constitutes 75-80% of the skeleton and is found in the shafts of long bones, and on the surface of all bones (2). It is composed of lamellae, concentrically arranged around a small central canal with a blood vessel, forming a Haversian system (also called osteon) (2). Inside the vertebrae and the epiphyses of the long bones, trabecular bone is found. Trabecular bone is a rigid meshwork of mineralized bone, where number, size and distribution of the trabeculae are arranged in order to optimize strength. It constitutes 20% of the total skeletal mass, but contributes with 65-70% of the total bone surface and is the most metabolically active part of the skeleton, acting as a calcium reservoir (2). Trabecular bone is superior in withstanding compressive stress, and therefore is the predominant bone in the vertebrae (1). At different sites in the skeleton, the proportion of cortical and trabecular bone vary. The distal forearm and femoral neck constitute 25% trabecular and 75% cortical bone, whereas the vertebrae contain more than two thirds trabecular bone (2). Loss of cortical bone and loss of trabecular bone hence predispose to fractures at different locations.

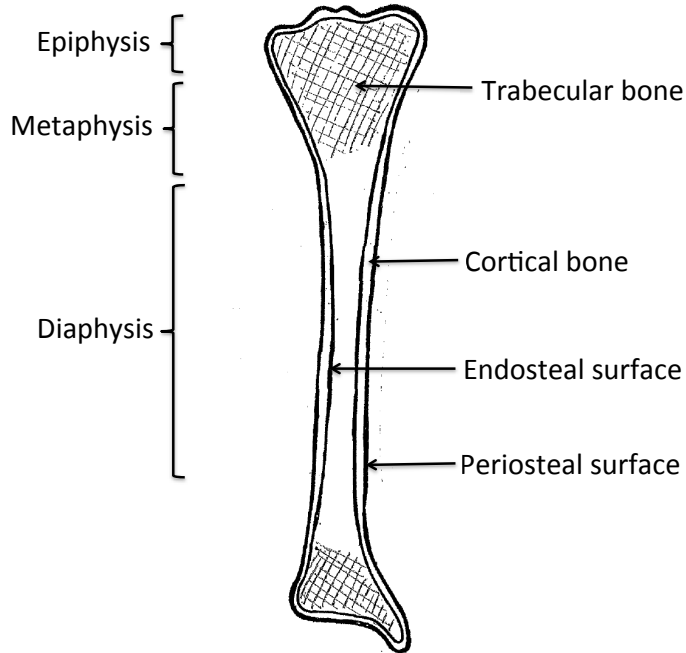


Figure 1. Schematic view of a longitudinal section through the tibia, a long (tubular) bone.

1.3 The bone cells

The skeleton consists of three different kinds of bone cells and of extracellular matrix (ECM). 90% of the total bone volume is ECM, which comprises mineralized matrix, organic matrix, lipids and water (3). Hydroxyapatite, $(Ca_{10}(PO_4)_6(OH)_2)^2$ is the main component of the mineralized matrix and provides the majority of bone strength and stiffness. The mineralized matrix accounts for 99% of the body's storage of calcium and 85% of the phosphorous (3). The organic matrix contains mainly type 1 collagen, as well as proteoglycans, growth factors, and glycoproteins. The organic matrix is secreted by osteoblasts and is mineralized within 10-15 days (3).

Osteoblasts

Osteoblasts are responsible for bone formation. They originate from mesenchymal stem cells in the bone marrow, and account for 4-6 % of the bone cells (4). Osteoblasts build bone by secreting bone proteins and collagen that form the bone matrix. Alongside collagen type 1, osteoblasts also

produce osteocalcin (OC), osteonectin, osteopontin and bone sialoprotein (4). The average lifespan of an osteoblast is three months (5). The aging osteoblast face three possible destinies: undergo apoptosis, become embedded in the bone as an osteocyte, or transform into a bone lining cell (6). The lining cells are flat cells located on top of a thin layer of unmineralized collagen matrix which covers the bone surface. When bone remodeling should not occur, they prevent direct interaction between osteoclasts and bone matrix (4). In order to allow osteoclasts to attach to the bone, the collagen matrix must be removed through collagenase secretion, and possibly the lining cells are responsible for this (5).

Osteocytes

When bone is formed, some osteoblasts become entrapped in the newly formed bone matrix and develop into osteocytes. Osteocytes account for approximately 95% of the bone cells, they are long-lived and do not divide (4). The osteocytes are located in lacunae and form a network via dendritic extensions into canaliculi, which are fluid-filled channels where the cells connect to each other. The osteocytes have the capacity to detect mechanical pressure and load (6), and thereafter regulate bone remodeling by acting on osteoblast and osteoclast differentiation and function (4).

Osteoclasts

Osteoclasts are the only cells that can resorb bone (7). They are essential for physiological bone resorption during growth, for remodeling, and for maintaining calcium homeostasis (8). Osteoclasts originate from haematopoietic stem cells, and are formed by fusion of several mononucleated cells resulting in large multinucleated cells (9). Osteoclast differentiation is promoted by the interaction between receptor activator of nuclear factor κ B (RANK) ligand, a cytokine expressed by osteoblasts, and RANK, expressed on osteoclasts (2). Mature osteoclasts can attach to the bone surface, creating an acidic microenvironment that enables bone resorption (10). After they have finished resorbing bone, osteoclasts undergo apoptosis (2).

1.4 Bone remodeling

Bone remodeling is the constantly ongoing process where bone is resorbed and formed (11). Just as roads, bridges and buildings, bone develop fatigue damage (12). Bone has the unique ability to detect micro-cracks or apoptotic osteocytes, remove the damaged bone, and replace it with new bone (13). The osteocytes are the cells that sense microcracks and mechanical strain and trigger bone remodeling (14). The remodeling cycle begins with recruitment

of osteoclasts for bone resorption and ends with bone formation by the osteoblasts, with bone formation lasting approximately three times as long as bone resorption (9). This process is completed in approximately 3-6 months (1), and takes place in a basic multicellular unit (BMU), consisting of bone resorbing osteoclasts, bone forming osteoblasts, osteocytes embedded in bone matrix, lining cells and the capillary blood supply (9). Bone remodeling takes place throughout the entire skeleton, and it has been estimated that the adult skeleton is completely regenerated in 10 years (estimated turnover 10% per year for the entire skeleton, considering cortical bone turnover on average 4% and trabecular bone turnover on average 28% per year) (5). At the cellular level, bone loss occurs because of an imbalance between osteoblast and osteoclast activity. For example, oestrogen deficiency after menopause leads to enhanced formation of osteoclasts, which causes increased bone turnover and progressive loss of trabecular bone (15). Remodeling imbalance causes reduced bone strength and can lead to osteoporosis (16).

1.5 Osteoporosis and its consequences

Osteoporosis is characterized by low bone density and microarchitectural deterioration of bone tissue, leading to an increased risk of fracture (17). The incidence increases with age, and at the age of 50, the remaining lifetime probability for a fragility fracture in Sweden is around 20% for men and 50% for women (18). The most common osteoporotic fractures are fractures of the vertebrae (spine), proximal femur (hip) and distal forearm (wrist) (19). Osteoporosis and its related fractures are a major public health issue. Patients with fractures (especially hip fractures but also vertebral fractures) suffer loss of quality of life and mobility, long-term disability and loss of independence (20). Only half of those ambulatory before a hip fracture are able to walk independently afterwards (21). Fractures also increase the mortality and impose a financial burden on the health care system (20). In men, osteoporotic fractures occur approximately 5-10 years later in life than in women, and after a hip fracture morbidity and mortality rates are higher among men (22). Osteoporosis is defined as bone mineral density (BMD) 2.5 standard deviations (SD) or more below the average of healthy young adult women (23). Low BMD is a major risk factor for osteoporotic fracture (24). Every SD decrease in BMD is associated with approximately a two-fold increase in the age-adjusted hip fracture risk in postmenopausal women, and with a three-fold risk increase in elderly men (24-26). Other important risk factors for osteoporotic fracture are increasing age, female sex, previous fracture, a family history of fracture, and systemic glucocorticoid treatment (27-29). Additional risk factors are smoking, excessive alcohol intake and

low body mass index (BMI), as well as increased risk of falling related to visual impairment, treatment with sedatives or reduced mobility (30-33). A few years ago, FRAX[®], a fracture risk assessment tool developed by the WHO, was introduced as a mean to identify patients with high fracture risk. It is a country-specific computer-based algorithm available on the internet, which calculates fracture probability based on risk factors and patient characteristics, with or without available measurements of femoral neck areal BMD (aBMD) (34).

1.6 Bone mineral accrual during growth

From early childhood to late adolescence, there is an ongoing accumulation of skeletal mass, which increases from approximately 70-95 g at birth to 2400 to 3300 g in young women and men, respectively (35). Skeletal growth requires adequate production of growth hormone, thyroid hormones, growth factors and sex steroids (36). Before puberty, skeletal growth is largely driven by growth hormone. Sex steroids are responsible for epiphyseal maturation and mineral accrual during adolescence (36). During childhood and adolescence, longitudinal growth as well as changes in skeletal size and shape occur (37). Bones become longer through endochondral ossification at the growth plates, and wider by periosteal apposition. During puberty, bone formation also occurs at the endosteal, inner surface of the cortical bone (37). Skeletal modeling during growth differs from bone remodeling, since it leads to new bone formation at a different location than the site of resorption, resulting in alterations in bone shape and net accrual of bone tissue (38). Before puberty, no substantial sex difference has been reported in bone mass of the lumbar spine, femur and radius, after adjustment for age, physical activity and nutrition (39). During puberty, bone size increases while volumetric BMD (vBMD) remains almost constant in both sexes (40). At the end of puberty, males have higher BMD, mainly due to greater bone size in males compared to females (37). In males, the prolonged bone maturation period results in larger increases in cortical thickness and bone size. Cortical thickness increases more by periosteal apposition of bone in males, resulting in a stronger bone, whereas in females more bone is deposited at the endosteal, inner surface (39). There is no significant sex difference in vBMD at the end of puberty (40, 41). A large part of the bone mineral content (BMC) accrual takes place during the period of adolescent growth surrounding peak height velocity (PHV), which is the time of the most rapid longitudinal growth. Depending on skeletal site, 33% to 46% of the adult BMC has been reported to be accrued in the circumpubertal years (-2 to +2

years from PHV) (42). Thus, this period of life is of great importance in order to optimize peak bone mass.

1.7 Peak bone mass

During childhood and adolescence, bone mass increases until a plateau is reached, the peak bone mass (PBM) (43). The timing of PBM has been debated, and differs between the sexes, between different skeletal sites, and also between different measurement methods (44-50), but PBM is generally believed to be reached in the late teenage years or young adult years (42). Achieving a high PBM is considered a key determinant of skeletal health throughout life. The bone mass of an individual later in life is a result of the peak bone mass accrued, as well as the subsequent rate of bone loss (15). It has been proposed that an increase in PBM of 10% (about 1 SD) could delay the onset of osteoporosis by 13 years, and could reduce the risk of fracture by 50% in postmenopausal women (37). Maximizing peak bone mass therefore seems to be of great importance in order to reduce the risk of osteoporotic fractures.

1.8 Determinants of peak bone mass

Heredity is the major determinant of PBM, and approximately 60-80% of the variance in PBM has been attributed to genetic factors (36, 51). Several environmental factors also influence PBM, among them nutritional intake, physical activity and smoking. Key nutritional factors are adequate intake of calcium and vitamin D. Association studies between calcium intake and BMC or BMD have been performed in children and adolescents, and most, but not all, suggest a positive association (52, 53). Several prospective randomized controlled trials with calcium supplementation have also demonstrated a positive effect on aBMD (53). However, in a review and meta-analysis of 19 studies, authors concluded that calcium supplementation in healthy children had no effect on aBMD at the hip or spine, but a small positive effect was seen on aBMD of the upper limb (54, 55). Few of the studies included in the review were performed in children with low baseline calcium intake. In another meta-analysis, authors concluded that increased dietary calcium intake significantly increased total body and lumbar spine BMC in children with low baseline calcium intake (56). Vitamin D is essential to bone mineralization because of its effects on intestinal calcium absorption and on bone mineral accrual (57). Vitamin D levels in the body are influenced by dietary intake, genetic factors, and endogenous synthesis in the skin following sunlight exposure, more specifically ultraviolet B (UVB)

irradiation (58, 59). In the northern hemisphere at latitudes greater than 40°N (north of Madrid), the sunlight is not strong enough to trigger vitamin D synthesis in the skin from October to March (59). In a review and meta-analysis of 6 studies, the authors concluded that it is unlikely that vitamin D supplements are beneficial for bone health in children and adolescents with normal vitamin D levels, but supplementation in deficient children and adolescents could result in clinically important improvements in bone density (60, 61). Insufficient serum vitamin D (25(OH)D) levels were recently demonstrated to be associated with low aBMD in Finnish children and adolescents age 7-19 years (62). In the Finnish study, only 29% of the 195 subjects had sufficient (>50 nmol/l) vitamin D status over the school year (62). The authors concluded that the findings may be representative of other Nordic and North European countries. Physical activity seems to be a key factor to increase bone mineral accrual during childhood and adolescence. The skeleton adapts to the forces and loads it experiences by increasing bone mass and remodeling in order to increase strength (63). Weight-bearing activities, ideally involving jumping, turning and sprinting, are preferred over non weight-bearing activities such as swimming or bicycling when it comes to increasing bone mass (64, 65). In children before and during puberty, positive effects of weight-bearing exercise on bone have been demonstrated in exercise intervention trials (63, 66-68). Recently, larger gains in BMD but no increased fracture risk was reported in a study of schoolchildren in Malmö, Sweden, taking part in daily physical education during six years compared to controls (200 min versus 60 min physical education weekly) (69). The authors concluded that a daily physical activity program ought to be implemented from school start for all children. Cross-sectional and longitudinal observational studies suggest that the beneficial effects of physical activity on bone persist into young adulthood (70-72). In the GOOD study, men who increased their physical activity between baseline (18-20 yrs) and follow-up (23-25 yrs) had an advantageous development of aBMD, trabecular vBMD, and cortical bone size (73), indicating that also in young adulthood, physical activity has a positive effect on bone development. Another lifestyle factor potentially influencing peak bone mass is smoking. Smoking has been associated with reduced bone mass and increased fracture risk in adult men and women (30, 74-76). Less is known about the effect of smoking at the time of peak bone mass accrual, but studies have demonstrated a negative relationship between smoking and BMD in both male and female adolescents (77-79). In the GOOD cohort, cross-sectional data showed that smoking was associated with lower aBMD of especially the femoral neck, and reduced cortical thickness of the radius and tibia at age 18-20 years (80), and longitudinal data demonstrated that smoking was associated with impaired bone mass development in young adulthood (81).

1.9 Pubertal timing and peak bone mass

Another variable suggested to influence peak bone mass is pubertal timing (39). Late menarche has been associated with low aBMD and compromised microstructure of the radius and tibia in young adult women (124 women, 20.4 ± 0.6 years (mean \pm SD)) (82, 83). Areal BMD at all sites was demonstrated to be inversely related to pubertal timing in teenagers of both genders, in a healthy cohort with variations in pubertal timing within the normal range (78 girls and 85 boys, 15.1 ± 1.0 and 16.2 ± 0.9 yrs at follow-up, respectively) (84). Results from another study showed lower BMC in late maturing males and females at 13-15 years of age, however, in late adolescence and young adulthood (17-28 years of age), no significant differences in BMC in early, middle, and late maturing men were seen, while late maturing females developed less bone mass throughout adolescence than their early and average maturing peers (85). In the GOOD study, pubertal timing in relation to BMD and bone size was investigated cross-sectionally (642 men, 18.9 ± 0.5 years), demonstrating that late pubertal timing was associated with lower aBMD at all measured sites, lower cortical and trabecular vBMD in both the radius and tibia, as well as reduced cortical thickness of the radius and tibia (86). In order to establish whether these deficits remain in adulthood, longitudinal studies are needed.

1.10 Fractures during growth

More than one in three children suffers a fracture during growth (87, 88), and boys have a higher risk than girls of sustaining a fracture in the first 16 years of life (89). It has been established that low aBMD is associated with prevalent fractures in children as well as in adults (90-94), and it has been suggested that fractures during childhood could be a predictor of low peak bone mass and persistent bone fragility (95). A recently published 27-year prospective longitudinal study demonstrated that in men, a childhood fracture was associated with low BMD and smaller bone size in adulthood (age 30-44 years), while in women, the deficit did not reach statistical significance (96). In contrast to this finding, there was no association between a self-recalled childhood fracture and future fractures in a large cohort study (97). The peak incidence of fractures is at the age of 11 to 12 years among girls and 13 to 14 years among boys (88, 90, 98, 99), which coincides with the age at peak height velocity, but fracture incidence has not previously been investigated directly in relation to PHV. Studies have demonstrated that age at PHV precedes the peak in bone mass accrual by approximately one year (100, 101). The delay in bone mass accrual in relation to linear growth has been suggested to cause a transient skeletal deficit in bone mass and

mineralization, increasing the susceptibility to fractures at this period in life (95, 102). Previous studies have mainly used dual energy X-ray absorptiometry (DXA) technique when assessing bone mass in relation to fractures in children and adolescents, and consequently it has not been clarified whether it is deficits in bone density or bone size that is the main contributor to fractures during growth.

1.1.1 Bone turnover markers

In recent years, attention has been drawn to the use of bone turnover markers (BTM) in the evaluation of osteoporosis treatment (103-106), and also as a determinant of fracture risk (107-109). Bone turnover markers are substances that reflect bone turnover and can be measured in samples of blood or urine. They can be divided into two categories: bone formation markers, which reflect osteoblast activity, and are byproducts of collagen synthesis, matrix proteins or osteoblastic enzymes; and bone resorption markers, which reflect osteoclast activity and are mainly degradation products of type 1 collagen (38). A common bone formation marker is osteocalcin (OC), produced by osteoblasts during osteoid synthesis (110). It is incorporated directly into bone matrix, but some newly synthesized and secreted OC circulates in blood (111). One of the bone resorption markers used is N-terminal telopeptide fragment (NTX), a collagen degradation fragment which, when bone is resorbed, is released into the blood and subsequently excreted in urine (112). It can be measured in serum (sNTX) or urine (uNTX). Several other markers of bone turnover are available. Bone turnover during growth can roughly be divided into four periods: infancy, prepubertal period, puberty and the postpubertal period, with corresponding changes in the levels of BTM (111). During puberty, levels of bone turnover markers increase, and have been demonstrated to be higher in early puberty and midpuberty compared to advanced puberty (113-115). If measurement of bone turnover markers can predict bone development around the time of peak bone mass has not previously been investigated, but is one of the foci of the present thesis.

1.12 Techniques for measuring bone density

1.12.1 Dual energy X-ray absorptiometry (DXA)

The DXA scanner was introduced in the late 1980s, and is the most widely used technique to assess bone density. In order to separate dense tissue from soft tissue, the DXA scanner produces two X-ray beams, one with high energy and one with low energy. For each beam, the amount of X-ray that passes through the body is measured, and bone density can be calculated. The radiation dose from a DXA examination is very low, ranging from 1-10 μSv for a spine and hip examination, which is comparable to the daily natural background radiation (116). The procedure is painless and noninvasive. In clinical use, DXA is primarily used to measure BMD of the lumbar spine and femoral neck. These measurements are used to diagnose osteoporosis, assess fracture risk, or monitor response to treatment. In postmenopausal women, the interpretation of the results are made in comparison to T-scores. A T-score equal to or less than -2.5 means that the BMD value lies 2.5 SDs or more below the average value in young adult women, and indicates osteoporosis according to the WHO classification of osteoporosis from 1994 (117). In younger individuals, Z-score is used instead of T-score. The Z-score represents the number of SDs that a given value for BMD deviates from the average value for the respective age and gender (118). Limitations with the DXA technique include that it renders two-dimensional projection images. The BMC of a given area is measured, and BMD (aBMD) is calculated by dividing BMC with area (g/cm^2). Thus, no consideration is given to the depth or volume of the bone, which means that a smaller bone can be falsely interpreted as having a low BMD. This is particularly a problem when measuring children (116). Measurement errors also occur because of the heterogeneity in soft tissue composition in different persons (accuracy error). The precision errors are smaller than the accuracy errors because when a person is rescanned, the effect of adipose tissue on the measurement is the same. Results and radiation dose can differ somewhat when using DXA scanners from different manufacturers (116).

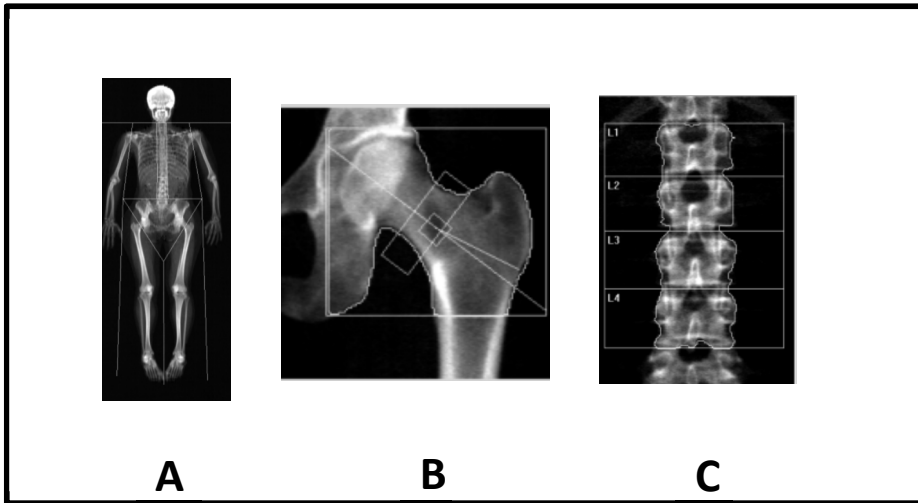


Figure 2. Images assessed by DXA (Hologic Discovery W). A) Total body B) Total hip C) Lumbar spine.

1.12.2 Peripheral quantitative computed tomography (pQCT)

Computed tomography is an X-ray based technique that provides three-dimensional information on morphology and composition of the scanned area. The peripheral quantitative computed tomography (pQCT) is a QCT device designed to measure peripheral bones, typically the distal forearm (radius) and the distal tibia (119). The radiation dose is low, less than 3 μSv for a single slice and increases in a multiple-slice protocol (120, 121). Unlike DXA, the pQCT renders cross-sectional images. This enables the separation of cortical and trabecular bone, so that vBMD of the cortical and trabecular bone compartments can be determined separately. It also allows for investigation of the geometrical bone properties. Information about trabecular bone traits are obtained through a scan at 4% of the bone length in the distal direction of the proximal end of the bone, while information about cortical bone traits is obtained through a scan at 15-65% of the bone length in the distal direction of the proximal end of the bone. Apart from vBMD of the trabecular and cortical bone, cortical thickness, cortical CSA, endosteal and periosteal circumference can be obtained, which enables us to study size and density of the bone separately.

1.12.3 High resolution pQCT (HR-pQCT)

Recently, a multislice high-resolution pQCT has come into use. A HR-pQCT is a pQCT device that performs several cross-sectional slices and produces a three-dimensional image of the bone. The resolution is high (slice thickness 82 μm) and enables investigation of the microstructure of the trabecular bone: the number of trabeculae, trabecular thickness, and how far separated the trabeculae are.

2 AIM

The general aim of the thesis was to study the development of bone density and bone geometry in young men around the time of peak bone mass, and to evaluate the association between pubertal timing and bone development in this period of life. In addition, the aim was to investigate whether bone turnover markers could predict bone development in young men, and also to establish if fracture during childhood and adolescence was related to bone density and bone geometry in young adulthood.

The specific aims of the papers included in the thesis were:

- I. To determine if it is the bone size or the vBMD that is most strongly associated with X-ray-verified prevalent fractures during childhood and adolescence in young men.
- II. To investigate the site-specific development of bone geometry, aBMD, and vBMD in young men during a five-year follow-up period, between the age of 19 and 24 years.
- III. To establish if pubertal timing is related to the development of bone geometry, BMC, aBMD, and vBMD in young men between the age of 19 and 24 years, and also to determine if late puberty is associated with remaining low bone mass in young adulthood in men.
- IV. To evaluate if baseline measurements of OC and NTX could predict the development of bone geometry, BMC, aBMD, and vBMD in young men between the age of 19 and 24 years.

3 MATERIALS AND METHODS

3.1 Subjects

All papers included in the thesis are based on the Gothenburg Obesity and Osteoporosis Determinants (GOOD) Study. The GOOD study is a population-based study with the aim to determine both environmental and genetic factors involved in the regulation of bone mass and fat mass. After being randomly identified using national population registers, subjects were contacted by telephone and asked to participate in the study. In order to be included in the GOOD study, subjects had to be between 18 and 20 years of age and willing to participate in the study. No other exclusion criteria were used. 48.6% of the contacted men agreed to participate and were included in the study. In total, 1068 men, 18.9 ± 0.9 years of age, from the greater Gothenburg area were included. The GOOD cohort was found representative of the general young male population in Gothenburg (48). Five years after the baseline visit, the men were contacted by letter and telephone and asked to participate in the follow-up study. 112 men could not be reached, and 107 men declined to participate, leaving 833 men included in the five-year follow-up study.

3.2 Anthropometrics and questionnaires

Height was measured with a wall-mounted stadiometer, and weight was measured to the nearest 0.1 kg. A self-administered questionnaire was used both at baseline and follow-up to collect information about nutritional intake, fracture history, and physical activity pattern. Calcium intake was calculated from reported dairy consumption. Blood samples were drawn and stored at -80°C until analysis.

3.3 Estimation of age at peak height velocity (PHV)

Out of the 1068 subjects included in the GOOD study, complete growth charts were available for 642 subjects. Age and BMI did not differ between the subset with available growth charts and the complete cohort, indicating that the subset is representative for the complete GOOD cohort (86). Growth charts were used for determination of age at peak height velocity according to the infancy-childhood-puberty (ICP) model. The ICP model isolates three distinct components of growth (infancy, childhood, and puberty) that can be

described using three different mathematical functions (122, 123). Age at PHV was defined as the age at maximum growth velocity during puberty, and was estimated by the algorithm. Age at PHV is the most common indicator of maturity used in longitudinal studies (85), and is generally believed to be reached within 2 years of pubertal onset (123, 124).

3.4 X-ray registers

In order to verify reported fractures, X-ray registers were searched. We searched the centralized computerized X-ray registers containing radiographs and reports from radiologists from 1991 onwards from the public hospitals in Gothenburg (Sahlgrenska, Mölndal and Östra (including Queen Silvia's children's hospital)). Computerized registers were also searched in the small private hospitals (Carlanderska and Lundby). A central archive containing X-ray reports from the entire Västra Götalandsregionen (a region encompassing a large part of southwestern Sweden with approximately 1.5 million inhabitants) of earlier dates was searched manually using social security numbers, as was the local archive of the Queen Silvia's children's hospital in Gothenburg. The fractures were classified according to skeletal site, based on the radiologist's report. No information concerning trauma severity was available. Subjects reporting fractures that could not be verified in the records were excluded from analysis.

3.5 Measurements of bone density

At both the baseline and the follow-up visit, the subjects underwent measurements with DXA and pQCT. At the follow-up visit, subjects also underwent examination with HR-pQCT (results not presented in this thesis).

Dual energy X-ray absorptiometry (DXA)

BMC (g), bone area (cm²), and aBMD (g/cm²) of the total body, femoral neck and total hip (of the left leg), and the left and right radius were assessed at baseline and follow-up using the Lunar Prodigy DXA (GE Lunar Corp. Madison, WI, USA). The Lunar Prodigy DXA used at the follow-up visit was not the same specimen used at the baseline visit. A cross-calibration between the two Lunar Prodigy DXA machines was performed at the time of follow-up and is described in detail in paper II. One person performed all the measurements of the baseline study, and another person performed all the measurements of the follow-up study. The CVs for the aBMD measurements ranged from 0.5-3%, depending on application.

Peripheral quantitative computed tomography (pQCT)

A pQCT device (XCT-2000; Stratec Medizintechnik, GmbH, Pforzheim, Germany) was used to scan the distal arm (radius) and the distal leg (tibia) of the nondominant arm and leg, respectively. A 2-mm-thick tomographic slice was scanned with a voxel size of 0.50 mm. The cortical vBMD (not including the bone marrow) (mg/cm^3), cortical cross-sectional area (CSA; mm^2), endosteal circumference (EC; mm), periosteal circumference (PC; mm) were measured using a scan through the diaphysis (at 25% of the bone length in the proximal direction of the distal end of the bone) of the radius and tibia. The threshold for cortical bone was $711 \text{ mg}/\text{cm}^3$. Polar strength strain index of the cortex (SSI; mm^3), was calculated by the software, version 6.00 of the XCT-2000. SSI represents an estimation of the mechanical strength of the cortical bone (125). Trabecular vBMD (mg/cm^3) was measured using a scan through the metaphysis (at 4% of the bone length in the proximal direction of the distal end of the bone) of these bones. Trabecular vBMD was assessed using the inner 45% of these bones. Tibial bone length was measured from the medial malleolus to the medial condyle of the tibia, and length of the forearm was defined as the distance from the olecranon to the ulna styloid process. Measurements at the follow-up visit were made using the same procedure and the same equipment as at the baseline visit. One person performed all the measurements of the baseline study, and another person performed all the measurements of the follow-up study. The CVs were less than 1% for all pQCT measurements.

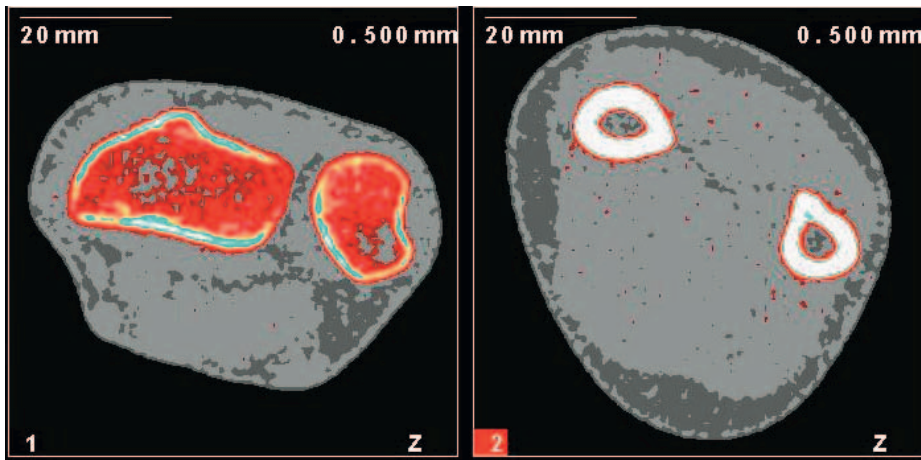


Figure 3. To the left, a scan through the metaphysis of the radius (and ulna) for measuring trabecular vBMD. To the right, a scan through the diaphysis of the radius (and ulna) for measuring cortical vBMD and cortical geometry. (XCT-2000; Stratec Medizintechnik, GmbH, Pforzheim, Germany)

3.6 Ethical considerations

The ethical considerations concerning the GOOD study included radiation exposure of study participants. Although DXA and pQCT are techniques with very low radiation, there is a small additional exposure. In the follow-up study, when participants were examined with DXA, pQCT and HR-pQCT, the effective radiation dose was estimated to 0.2 mSv, which corresponds to approximately 2 months of background radiation. At baseline, the dose was even lower since only DXA and pQCT measurements were performed. Venous blood sampling is an invasive procedure, but with minimal risks of complications. There is also the question of personal integrity. The data was collected in a database where personal data only existed in coded form, and consequently no connection between particular individuals and data could be made. Only authorized persons had access to the database. The results have been presented on a group level, so that no individual can be identified. Participants could withdraw from the study at any point. Written and oral informed consent was obtained from all included subjects. The regional ethical review board at the University of Gothenburg approved all studies included in the thesis.

3.7 Statistics

Differences in anthropometric characteristics and bone parameters in paper I (fracture/non-fracture) were investigated using an independent samples t-test, in paper II (baseline/follow-up) using a paired-samples t-test, and in paper III (early/middle/late puberty) and IV (quartiles of OC level) using one-way ANOVA followed by Bonferroni post hoc test. Chi-square test was used in all papers to compare categorical variables. To evaluate the association between prevalent fracture and bone variables (paper I) and age at PHV (paper III), binary logistic regression analysis was performed. Linear regression equations including covariates height, weight, smoking, calcium intake and physical activity were performed in order to calculate adjusted baseline and 5-year bone variables and the 5-year changes (also adjusted for follow-up time) in bone variables (paper II). To determine which variables were independent predictors of the change over 5 years in different bone variables, stepwise multiple linear regression analyses were performed (paper III-IV). The stepwise selection process criterion for entry into the model was a p-value ≤ 0.05 , and the criterion for removal from the model was a p-value ≥ 0.10 . The percentage of the variation (R^2) of the change over five years in each bone parameter explained by different variables was calculated using the stepwise linear regression model (paper III-IV). Bivariate correlations were calculated using Pearson's correlation (paper II and IV). Parameters that

did not display a normal distribution were logarithmically transformed before being entered into the regression analysis. In paper IV, a spline regression model was used to study the linearity of the association between OC and changes in BMD in more detail. A p value less than 0.05 was considered significant. Data was analyzed using SPSS software for Windows (paper I, version 16.0, paper II version 15.0 and 19.0, paper III version 19.0, paper IV version 20.0).

Statistical methods used:	Paper I	Paper II	Paper III	Paper IV
Independent samples t-test	x			
Paired samples t-test		x		
ANOVA and post hoc test			x	x
Chi square test	x	x	x	x
Binary logistic regression	x		x	
Linear regression		x	x	x
Bivariate correlation		x		x
Log transformation	x	x	x	x
Spline regression model				x

Table 1. The different statistical methods used in papers I-IV.

4 RESULTS AND DISCUSSION

4.1 Paper I

Trabecular volumetric bone mineral density is associated with previous fracture during childhood and adolescence in males: the GOOD study

In the first study in the thesis, we investigated the association between fracture during childhood and adolescence and aBMD, vBMD and bone geometry in young adulthood. 304 men were identified as having had at least one previous X-ray-verified fracture, while 687 men were non-fracture subjects.

4.1.1 Main results

- Men with prevalent fracture had
 - lower aBMD than men without fracture at all measured sites (total body, lumbar spine, total hip, radius).
 - lower cortical and trabecular vBMD of the radius and tibia, as well as slightly reduced cortical thickness of the radius due to a larger endosteal circumference.
- Trabecular vBMD of the radius was most strongly associated with previous fracture (radius fracture as well as any fracture).
- Among distal forearm fractures, fracture of the nondominant arm (mostly the left arm) was clearly overrepresented.
- Fracture incidence was shown to reach its peak at age at PHV, followed by a sharp decline thereafter (fig 4).

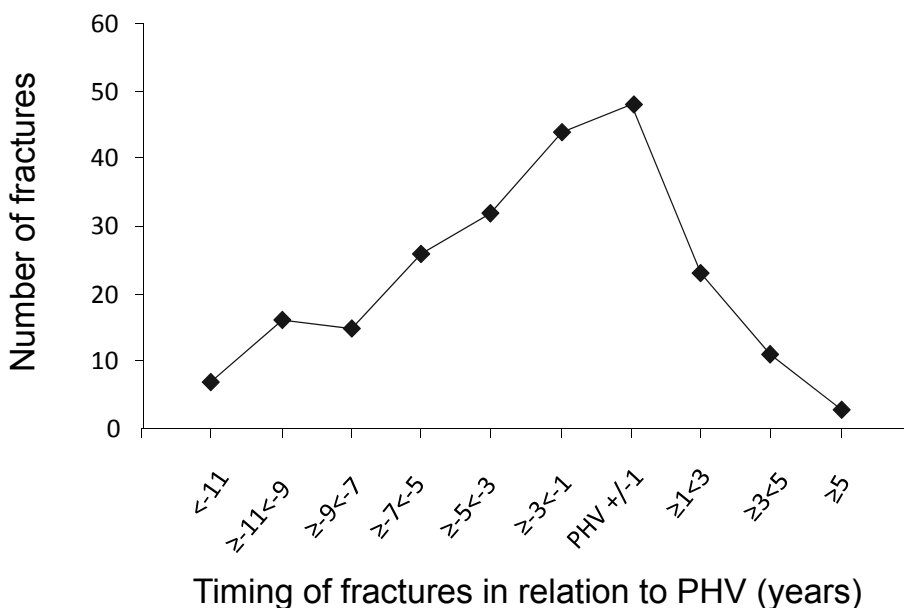


Figure 4. Fracture incidence according to PHV. Fracture incidence increased with advancing age and reached its peak at the time of PHV, followed by a sharp decline thereafter. From Darelid et al, JBMR (126). Reprinted with permission from the publisher.

4.1.2 Conclusion

In conclusion, previous fracture was associated with lower aBMD at all measured sites and lower vBMD, especially trabecular vBMD of the radius and tibia.

4.1.3 Discussion

304 out of 991 men (30.7%) of the men had experienced a fracture during growth, which corresponds to other reports of fracture incidence in males in this age group (88). When we analyzed the distribution of forearm fractures, we found that fracture of the nondominant arm (mostly the left arm) was clearly overrepresented. In a previous study of the same cohort, aBMD was demonstrated to be higher in the dominant arm than in the nondominant arm, indicating that lower aBMD of the nondominant arm may have contributed to this distribution (127). Fracture incidence was shown to reach its peak at age at PHV, followed by a sharp decline thereafter (fig 4). Several earlier studies have demonstrated a peak in fracture incidence of approximately 11-12 years for girls and 13-14 years for boys (88, 98, 128), which coincides with age at PHV. To the best of our knowledge, this is the first study ever to investigate fracture incidence in direct relation to age at PHV. Our findings indicate that

the skeleton is most susceptible to fracture when longitudinal growth is at its peak, which corresponds well to earlier findings that body size-corrected aBMD decreases to its minimum during age at PHV (102). Our results suggest that a reduction in trabecular vBMD, possibly in combination with reduced cortical thickness, could be responsible for this reduction in aBMD at the time of PHV. Taes and colleagues presented similar results in a study of adult men (25-45 years), although in that study cortical thickness was more strongly associated with childhood fracture than trabecular vBMD (129). In younger boys (15.2±0.5 years (SD±mean) at follow up), Chevalley et al demonstrated an association between fracture and lower femoral neck aBMD and lower trabecular vBMD of the tibia, due to a reduction in trabecular number as measured with HR-pQCT (130). The same authors reported that fractures were associated with lower aBMD and lower trabecular vBMD of the radius, as well as reduced trabecular thickness in young adult women (20.4±0.6 years, mean±SD), findings in concordance with the present study (131). Farr and colleagues concluded in a study of 465 girls 8-13 years old, that previous fracture was associated with lower trabecular vBMD, but not bone size, of the femur and tibia (132). In the five-year follow-up of the GOOD study, Rudäng et al investigated the association between childhood fractures and trabecular bone microstructure. Men with a childhood fracture (fracture ≤16 years of age) had lower trabecular bone volume fraction at both the radius and tibia, mainly due to smaller trabecular thickness but also increased trabecular separation, and at the radius also reduced trabecular number (133). The assumption that fractures in childhood may predict low peak bone mass was recently supported in a 27-year prospective study in men (96). In order to improve fracture risk prediction and develop prevention strategies, increased knowledge about the etiology of bone fragility is valuable.

4.2 Paper II

Cortical consolidation due to increased mineralization and endosteal contraction in young adult men: a five year longitudinal study

The second study was a longitudinal study investigating the normal development of aBMD, vBMD and bone geometry in young adulthood in men. Out of the 1068 men originally included in the GOOD study, 833 men agreed to participate in the five-year follow-up study.

4.2.1 Main results

- Areal BMD of the total body, lumbar spine and radius increased by 3.4%, 4.2% and 7.8%, respectively, over the five-year period.
- Areal BMD of the total hip and femoral neck decreased by 1.9% and 3.6%, respectively.
- Both cortical and trabecular vBMD of the radius increased, as well as cortical thickness of the radius due to a decreased endosteal circumference (fig 5). At the tibia, similar results were seen except for trabecular vBMD which decreased slightly. There was a suggested decrease in periosteal circumference of the tibia.
- The men included in the follow-up study were substantially heavier and slightly taller than at baseline five years earlier. Their reported calcium intake and weekly amount of physical activity were reduced compared to the baseline visit.

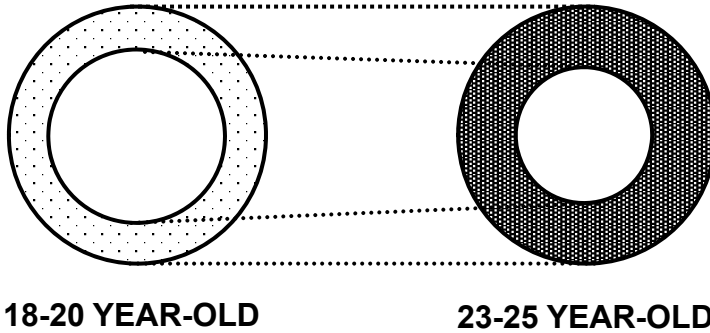


Figure 5. Increased mineralization and increased cortical thickness due to diminished endosteal circumference in young men between age 18-20 and 23-25 years. From Ohlsson and Darelid et al, JCEM (134). Reprinted with permission from the publisher.

4.2.2 Conclusion

In conclusion, aBMD decreased at the hip but increased at the spine and radius, and the increment in the long bones was explained by increased vBMD and increased cortical thickness due to endosteal contraction.

4.2.3 Discussion

Not many large, population-based longitudinal studies around the time of peak bone mass in males have been performed. To the best of our knowledge, the present study is the only one using pQCT, which enables us to elucidate whether change in density or size of the long bones explains the changes in aBMD during this period in life. In a cross-sectional analysis of the GOOD cohort, Lorentzon et al demonstrated that age was not correlated to aBMD of the lumbar spine, femoral neck and total body in men between 18-20 years, results that suggested that PBM had been reached at these sites (48). Radius aBMD was correlated to age, as was cortical thickness, EC and cortical vBMD of the radius, suggesting ongoing mineralization and increase in bone size in the long bones at 18-20 years of age (48). Results from the present study show that peak bone mass had been reached in the hip region, since aBMD already started to decrease in men between 19 and 24 years. In a longitudinal study including 527 men (16-40 years old), authors concluded that peak bone mass at the hip occurred between 19 and 21 years of age, findings relatively consistent with the present study (135). Similar results, with the peak in hip BMC observed at approximately 18.5 yrs (and for

femoral neck BMC 16.5 years) was reported by Baxter-Jones et al in a study with a mixed longitudinal design (42). Yet earlier, at approximately 16 years, was the estimated PBM of aBMD at the hip in a longitudinal study by Bachrach et al (136). Nilsson et al demonstrated that a reduced level of physical activity between baseline and follow-up was associated with greater loss of hip aBMD in the GOOD cohort (73). We hypothesize that weight-bearing bones are more dependent on continued physical activity in order to minimize bone loss. Study participants in the GOOD cohort who started to smoke between baseline and follow-up also lost more in total hip and femoral neck aBMD (81). There was an increase of 4.2% in lumbar spine aBMD between baseline and follow-up, suggesting that in the GOOD cohort, peak bone mass was not reached before 18 years of age but between 18-25 years or later. Lumbar spine PBM was reported to occur between 19-33 years of age in the aforementioned study by Berger et al (135), and the peak in lumbar spine BMC was at approximately 18.5 years in the study by Baxter-Jones et al (42). In the cohort investigated by Bachrach et al, lumbar spine peak aBMD was already reached by approximately 18 years (136). Also aBMD of the radius continued to increase (on average 7.8%) between 19 and 24 years in the GOOD cohort, and further investigation with pQCT elucidated that this increase was due mainly to increased cortical vBMD and greater cortical thickness as a result of reduced endosteal circumference. Thus, the process suggested in the cross-sectional analysis of the GOOD cohort at 18-20 years was confirmed, and continued until 23-25 years of age and possibly longer. A longitudinal study including 88 males (22-39 years at baseline) demonstrated increases in aBMD of the radius during a 4-year follow-up period (137), and a Norwegian population-based longitudinal study using single X-ray absorptiometry to investigate distal radius estimated peak bone mass in males to 34 years (138). Those results support the conclusion that PBM has not yet been reached at the radius in the GOOD cohort. In summary, peak bone mass appears to be reached at the hip region already before or around 18 years of age, followed by the spine in late teenage years or in the twenties, and in the long bones well after that. In the vast majority of studies, females reach PBM before males at all sites.

4.3 Paper III

Catch up in bone acquisition in young adult men with late normal puberty

In the third study, we investigated longitudinal development of aBMD, BMC, vBMD and bone geometry in relation to age at PHV. 501 men with available data on age at PHV participated in the baseline and follow-up study and were included in this study. We knew from previous results that men with late (but within the normal range) puberty had lower aBMD at age 18-20 years. The cohort was divided into tertiles according to age at PHV. Our aim was to determine if this deficit persisted into early adulthood, and to investigate whether pubertal timing influenced bone development in young adulthood.

4.3.1 Main results

- Men with late puberty
 - had significantly larger increases in aBMD and BMC of the total body, lumbar spine and radius than men with early puberty.
 - lost less in femoral neck aBMD.
 - gained significantly more in cortical and trabecular vBMD of the radius, as well as in cortical CSA and PC of the radius.
- Age at PHV was an independent positive predictor of
 - the increase in aBMD and BMC of the total body, lumbar spine and radius (fig 6).
 - the increase in trabecular and cortical vBMD of the radius.
 - the increase in cortical CSA and thickness of the radius.

- At age 23-25, no significant differences were seen between men in the different pubertal groups at the measured bone sites except for radius aBMD, which was still lower in men with late puberty due to lower cortical and trabecular vBMD.

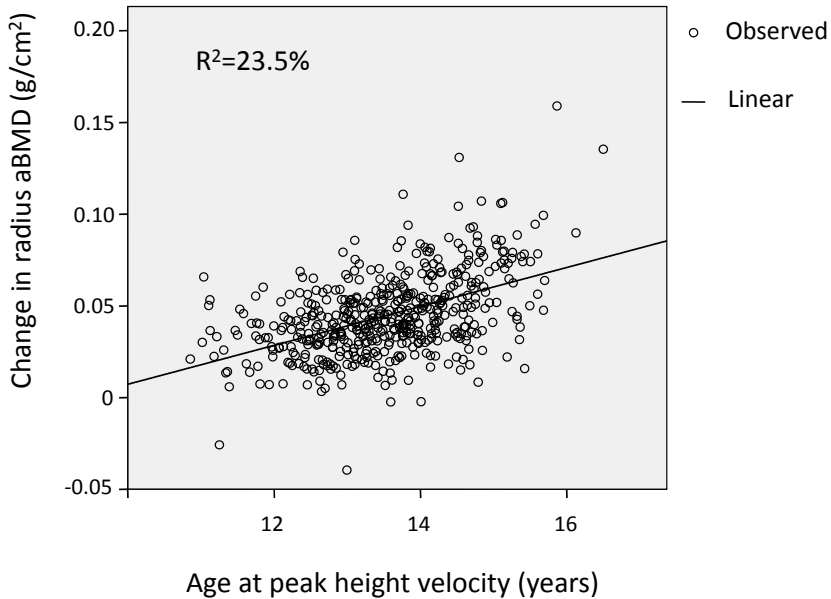


Figure 6. Age at PHV predicts increase in radius aBMD between 19 and 24 years. Higher age at PHV is associated with a larger increase in radius aBMD between 19 and 24 years of age. From Darelid et al, JBMR (139). Reprinted with permission from the publisher.

4.3.2 Conclusion

In conclusion, our results demonstrate that late puberty within the normal range was associated with a substantial catch up in aBMD, BMC, vBMD and bone size in young adulthood. No significant differences in aBMD or BMC of the lumbar spine, femoral neck or total body were seen at age 24 in men with late puberty, whereas aBMD of the radius was still significantly lower in men with late pubertal onset, due to lower cortical vBMD but not reduced bone size.

4.3.3 Discussion

The relationship between pubertal onset and adult bone mineral density has been mainly studied in women. Studies have shown that late menarche was associated with low aBMD and higher fracture risk at several sites in postmenopausal women (140-144), whereas early menarche was associated with high aBMD in premenopausal women (145, 146). In more recent studies by Chevalley and colleagues, an inverse relationship between aBMD of the radius and femoral neck and menarcheal age in young women (20.4 ± 0.6 years, mean \pm SD) and in premenopausal women (45.8 ± 3.4 years, mean \pm SD) was observed (82, 83). In the present study investigating changes between 18-20 and 23-25 years, no significant differences between men with early, middle and late puberty in aBMD of the lumbar spine, total hip, femoral neck and total body were seen at follow-up (23-25 yrs). Possibly, the effects of late puberty on adult BMD and BMC differ in men and women. This assumption is supported by the earlier mentioned study presented by Jackowski et al, where no significant differences in BMC in early, middle, and late maturing men were seen in late adolescence and young adulthood (17-28 years of age), while late maturing females developed less bone mass throughout adolescence than their early and average maturing peers (85). In the present study, there were no significant differences in bone geometry at 23-25 years of age, while cortical vBMD of the radius was significantly lower in men with late puberty. In women, Chevalley et al demonstrated lower cortical density and lower cortical thickness of the radius in young adult women with late menarcheal age (83). Due to the longitudinal design of our study, we could also investigate the relationship between pubertal timing and bone development between 18-20 years and 23-25 years. In most bone variables, men with late puberty had larger increases than men with early puberty, and we concluded that there is a notable catch up effect in bone acquisition during this period of life in men with late puberty. Nevertheless, the lower BMD observed in teenagers with late puberty has clinical relevance, since this is a period of high fracture incidence. In the present study, 43 out of 155 subjects (27.7%) with early puberty had experienced at least one fracture during growth or young adulthood, compared to 56 out of 147 (38.1%) men with middle, and 62 out of 154 (40.3%) men with late puberty ($p=0.049$). We also observed a 28% increased risk of having had a fracture during growth and young adulthood for each year age at PHV increased. Chevalley et al demonstrated even larger increases in the risk of having had a fracture with increasing age at menarche in young women (131).

4.4 Paper IV

In the final study of the thesis, we investigated whether baseline measurements of bone turnover markers could predict bone development in young men between age 19 and 24 years. 817 men who took part in the baseline and follow-up visit, and had a baseline value of osteocalcin and NTX, were included in the present study. The men were divided into quartiles according to level of OC.

4.4.1 Main results

- Men with a high OC level at baseline
 - had significantly larger increases in aBMD and BMC of the lumbar spine compared to men with a low OC level at baseline.
 - lost less in total hip aBMD and in trabecular vBMD of the tibia.
 - gained significantly more in trabecular vBMD of the radius, as well as in cortical CSA of the radius and tibia.
- Osteocalcin was an independent positive predictor of the increase in BMD and BMC of the total body, lumbar spine and radius.
- A high OC level at the age of 19 predicted a favorable development in BMC, aBMD (fig 7), vBMD and bone size between 19-24 years of age.

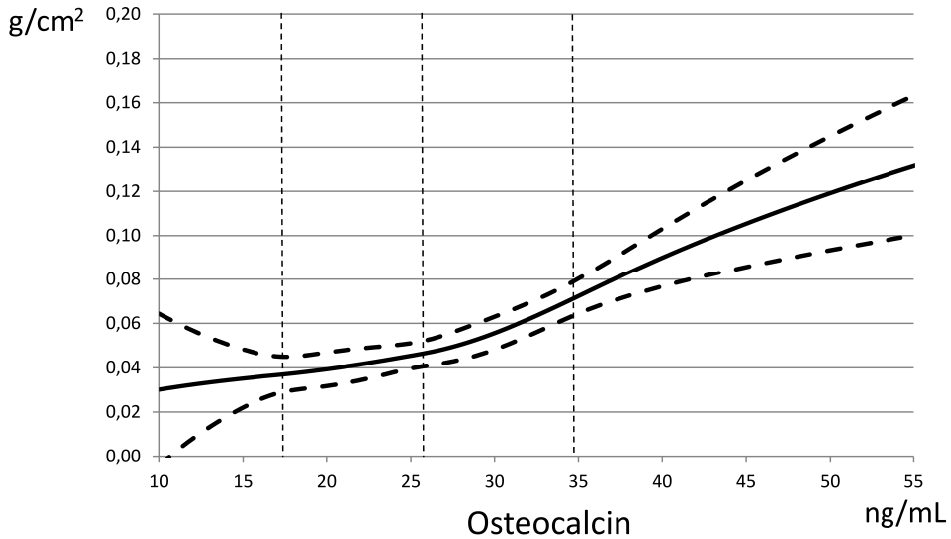


Figure 7. Change in lumbar spine aBMD between 19 and 24 years of age according to level of OC at baseline. From Darelid et al, JCEM (147). Reprinted with permission from the publisher.

4.4.2 Conclusion

In conclusion, our results demonstrate that high levels of OC at age 19 was associated with larger increases in BMC, aBMD, vBMD and bone size in young adulthood, indicating that measuring OC could be of value in the evaluation of bone development in a young male.

4.4.3 Discussion

In this final study of the thesis, we demonstrated that OC, measured at baseline, was an independent positive predictor of bone development in young men between 19 and 24 years. In paper II, we reported increases in aBMD of the lumbar spine, total body and radius, as well as decreased aBMD of the femoral neck and total hip in this cohort (134), indicating that this period in life is of importance in maximizing PBM. In paper III, age at PHV was demonstrated to be a positive predictor of bone development between 19 and 24 years in a subsample of men from the GOOD cohort (139). Age at PHV is an objective measurement of pubertal timing, and has been shown to be strongly correlated to age at menarche in females (148). Data to calculate age at PHV are generally not available in the clinical setting. When we divided the men into quartiles according to OC level at baseline, we found that men with high (35.2 ± 4.4 ng/ml, mean \pm SD) OC were slightly younger and taller, weighed less, and had experienced age at PHV on

average one year later than men with low (17.7 ± 2.3 ng/ml, mean \pm SD) OC at baseline. In previous studies, markers of bone turnover have been demonstrated to correlate well with age at PHV (149, 150). One small study ($n=100$) reported a very close correlation between OC levels and the pubertal growth spurt (151). We suggest that a young man with high OC at age 19 is at a lower maturational level and therefore may increase more in bone density, bone mass and bone size, than a man with low OC at this time in life. The correlation coefficient for age at PHV and log OC was 0.38 ($p < 0.001$), indicating that measuring OC could provide some information about the age at PHV and the bone maturational level. Longitudinal studies evaluating levels of BTM in relation to bone development are scarce. One five-year longitudinal study of 240 men (35-69 years) demonstrated that NTX, measured at follow-up, was negatively correlated with the change in aBMD at the femoral neck ($r = -0.21$) and could explain 3.8% of the variance of the change in femoral neck aBMD (152). In the present study, NTX was positively correlated to change in femoral neck BMC ($r = 0.13$) but not aBMD, and could not explain any of the variance of the change in femoral neck BMC or aBMD. OC, on the other hand, was positively correlated to the change in femoral neck BMC and aBMD ($r = 0.18$), and could explain 2.9% of the variance of the change in femoral neck aBMD.

4.5 General discussion

The two main components influencing an individual's risk of osteoporosis are PBM, achieved by young adulthood, and the subsequent rate of bone loss later in life (53). It has been proposed that osteoporosis should be considered "a pediatric disease with geriatric consequences" (35). Although a large part of the variation in PBM is genetically determined, it is also influenced by environmental factors. Achieving optimal PBM requires adequate hormone levels, proper nutrition (including sufficient intake of calcium and vitamin D), and loading of the skeleton through weight-bearing physical activity. The trend of increasing physical inactivity among contemporary youth is a concern for overall health (153-155), including the acquisition of optimal peak bone mass (156). Increased knowledge about the timing of PBM, as well as factors that influence the acquisition of PBM, are valuable in order to promote lifestyle choices that support the achievement of optimal PBM, which could reduce the risk of osteoporosis later in life. In this thesis, we present data from the GOOD study, a longitudinal study of young men around the time of PBM. Our results suggest that PBM was reached in the hip region in the late teens or early twenties, at the lumbar spine in the mid-twenties, and in the long bones later than mid-twenties. Having had a fracture

during childhood or adolescence was associated with lower BMD, indicating that a previous fracture is a risk factor for low BMD in young adulthood in males. The peak incidence in fractures coincided with age at peak height velocity, supporting the notion that this period in life confers a transient skeletal fragility due to a discrepancy in longitudinal growth and mineralization. Men with late pubertal timing had lower BMD at 18-20 years of age, but experienced larger gains in the following 5-year-period, resulting in a catch up effect. At 23-25 years of age, there was still a deficit in radius aBMD in men with late puberty, due to lower vBMD. We do not know if this deficit will persist into old age, or if the men with late puberty will increase further in vBMD of the long bones in the next few years. It appears as if late puberty has more substantial consequences for BMD in women than in men, but more studies are needed in order to clarify this. Possibly, the prolonged growth period in boys compared to girls contributes to lessen the effect of late puberty on PBM in males. We also showed that a high level of OC at age 18-20 was associated with larger gains in BMD and bone size in the following five years. Possibly, measuring OC could be of help when evaluating BMD in a young male, to get an indication of whether bone density will continue to increase or if that process has ceased. Further studies are needed in order to confirm this. A cross-sectional study performed within the GOOD cohort showed that men involved in physical activity more than 4 hours/week had higher aBMD of the lumbar spine and femoral neck, higher trabecular vBMD, and greater cortical thickness of the tibia, than men who were sedentary (127). Subjects who started their present amount of physical activity (minimum 4 hours/week) before age 13 had higher aBMD of the femoral neck, cortical CSA of the tibia, and trabecular vBMD of the tibia, than subjects who began at age 13 or later, suggesting that exercise before and during puberty is important for the acquisition of peak bone mass (127). When followed longitudinally, men in the GOOD cohort who increased their physical activity between baseline and follow-up had larger increases in aBMD of the lumbar spine and total body (73). They also had a small increase in hip aBMD, while men who reduced their level of physical activity had a decrease in hip aBMD (73). These results indicate that also in young adulthood, physical activity is of importance to optimize peak bone mass. In a cross-sectional analysis comparing smokers to non-smokers in the GOOD cohort, smokers had lower aBMD of the total body, lumbar spine and femoral neck, as well as reduced cortical thickness of the radius and tibia due to larger endosteal circumference (80). Men who started to smoke between baseline and follow-up gained less in aBMD of the total body and lumbar spine than their nonsmoking peers (81). They also lost more in hip aBMD and in trabecular vBMD of the tibia, indicating that smoking impairs bone development in adolescence and young adulthood.

Osteoporosis is a major public health issue, and the burden both economically and in terms of individual suffering will most likely increase in the years to come. A growing number of people will be getting osteoporosis due to increased longevity and a sedentary lifestyle. In children, adolescents and young adults, we can promote the achievement of a high peak bone mass by ensuring adequate calcium intake, supplementing vitamin D deficiency, and encouraging a non-smoking lifestyle with daily physical activity, thereby reducing the risk of osteoporosis and fractures later in life.

5 CONCLUSION

In a large cohort of young Swedish men investigated by DXA and pQCT, we found that a previous fracture was associated with lower aBMD at age 19, and especially with reduced trabecular vBMD of the radius. Between age 19 and 24 years, femoral neck aBMD decreased while lumbar spine aBMD increased. Radius aBMD increased, due to increased cortical thickness and continuing mineralization. Men with late puberty had larger gains in aBMD, vBMD, and bone size, and lost less in aBMD of the femoral neck, reflecting a catch-up in bone acquisition in young adulthood in men with late puberty. A high level of OC at age 19 was associated with larger gains in aBMD, vBMD, and bone size between 19 and 24 years of age. Even though the majority of bone acquisition takes place in the circumpubertal years, this thesis indicates that young adulthood is also a period of importance in order to attain optimal PBM, which in turn could be crucial for the prevention of osteoporosis and fragility fractures later in life.

RELATED PUBLICATIONS NOT INCLUDED IN THE THESIS

Rudäng R, Darelid A, Nilsson M, Nilsson S, Mellström D, Ohlsson C, Lorentzon M. Smoking is associated with impaired bone mass development in young adult men: A 5-year longitudinal study. *The Journal of Bone and Mineral Research*, October 2012;27(10):2189-2197.

Rudäng R, Darelid A, Nilsson M, Mellström D, Ohlsson C, Lorentzon M. X-ray verified fractures are associated with finite element analysis derived bone strength and trabecular microstructure in young adult men. *The Journal of Bone and Mineral Research*, November 2013;28(10):2305-2316

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